Standard Operating Procedure for Total Phosphorous in Sediments by Persulfate Oxidation Digestion (Lachat Method)

LG600

Revision 03, August 2004

Standard Operating Procedure for Total Phosphorous in Sediments by Persulfate Oxidation Digestion (Lachat Method)

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of total phosphorous in sediment samples.

1.2 The approximate working range for analysis is 0.1 to 10.0 mg P/L.

2.0 SUMMARY

2.1 Homogenous sediment samples which have been dried and ashed are digested in the presence of sulfuric acid and ammonium persulfate to hydrolyze polyphosphates and organic phosphorous into ortho-phosphate.

2.2 The orthophosphate ion $(PO_4)^{3-}$ reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form 12-molybdophosphoric acid. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

3.0 SAMPLE COLLECTION AND HANDLING

3.1 Sediment samples are collected from the lake floor and stored in sulfuric acid washed 500-mL plastic bottles. Samples should be kept refrigerated.

4.0 INTERFERENCES

4.1 Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant. A silica concentration of 50 mg SiO_2/L is required to produce a 0.008 mg phosphorous per liter positive error in orthophosphorous.

4.2 High concentrations of ferric iron or arsenate can cause error due the competition of the complex for ascorbic acid.

4.3 Since sediment samples may contain phosphorous in suspension combined with organic matter, it important that this organic matter is sufficiently oxidized during digestion to rupture both C–P and C–O–P bonds.

5.0 APPARATUS AND SUPPLIES

5.1 50-g capacity porcelain drying dishes

5.2 Mortar and pestle

5.3 Desiccator

5.4 60-mL glass digestion tubes with plastic Teflon-lined caps

5.5 Drying oven

5.6 Ash furnace

5.7 Autoclave

5.8 Automatic pipet, calibrated to 8.0 mL

5.9 Plastic disposable pipet tips

5.10 Lachat QuikChem AE auto-analyzer

5.10.1 Phosphate manifold (Lachat Method # 10-115-01-1-E)

5.10.2 Dot matrix printer

5.10.3 XYZ Sampler

6.0 REAGENTS AND STANDARDS

6.1 All reagent and standard storage containers must be labeled with the following information:

Reagent Identity:	(e.g., 20% Sulfuric acid)
Date of Preparation:	(e.g., 21DEC97)
Expiration Date:	(e.g., 21JAN98)
Initials of Preparer:	(e.g., IMF)
Concentration:	(e.g., 200 mL H_2SO_4/L)

6.2 All reagents and standards must be prepared using reagent water.

6.3 **1:1 Digestion Solution:** In a 0.5-L Erlenmeyer flask, stir together equal volumes of stock solution **6.3.1** and stock solution **6.3.2**. Cover the mouth of the flask with parafilm until use. This mixture should be prepared fresh for each digestion. The unused portion of the digestion solution should be disposed of in the yellow acid waste stream.

6.3.1 **Sulfuric Acid Digestion Stock:** To a 1-L volumetric flask add 500 mL reagent water and 250 mL H_2SO_4 (conc.). Stir and cool to room temperature. Dilute to volume and store in a plastic container at room temperature.

***NOTE**: This solution is extremely hot and very caustic to the skin. Be extremely caution when handling the flask. Wear oven mitts over acid resistant polypropyl gloves.*

6.3.2 **Ammonium Persulfate Digestion Stock:** To a 250-mL volumetric flask containing 200 mL reagent water, add 300 g Ammonium Persulfate. Stir the crystals until fully dissolved and dilute to the mark. Store in a plastic container in refrigerator. Prepare fresh every fifteen days.

6.4 **Color Reagent:** In a 1-L volumetric flask containing 300 mL reagent water, add 20.9 mL concentrated sulfuric acid, 72 mL stock **6.4.1**, and 213 mL stock **6.4.2.** Stir and dilute to the mark. Store in a plastic container in refrigerator. Degas with helium. Prepare this solution fresh each month.

NOTE: *Be sure that the conductivity of the laboratory reagent water is not elevated. This usually indicates that silica is not being properly filtered from the reagent water system. Elevated concentration of silica will cause a greenish blue turbid discoloring of the overall color reagent.*

6.4.1 **Antimony Potassium Tartrate Stock:** In a 1-L volumetric flask containing 250 mL reagent water, add 3.0 g antimony potassium tartrate. Stir to dissolve and dilute to the mark. Store in dark plastic at 4°C.

6.4.2 **Ammonium Molybdate Stock:** In a 1-L volumetric flask containing 500 mL reagent water, add 40.0 g ammonium molybdate tetrahydrate crystals. Stir to dissolve and dilute to the mark. Store in dark plastic container at 4°C.

6.5 **Ascorbic Acid Reducing Solution:** In a 1-L volumetric flask containing 850 mL reagent water, add 60.0 g L-ascorbic acid. Stir to dissolve. Degas with helium. Add 2.0 g dodecyl sulfate sodium salt, dissolve and dilute to the mark. Slowly pour this solution into a dark plastic container. Store at 4°C. Prepare fresh weekly.

6.6 **Carrier Solution:** To a 2-L volumetric flask containing 1500 mL reagent water, add 18.0 mL concentrated sulfuric acid. Mix and dilute to volume. Store in plastic at room temperature. Degas with helium.

6.7 **10 % Sulfuric Acid Cleansing Solution:** To a 1-L volumetric flask containing 750 mL reagent water, stir in 100 mL H_2SO_4 (conc.). Dilute to volume. Store in plastic at room temperature.

6.8 **Sodium Hydroxide - EDTA Rinse:** To a 1-L volumetric flask containing 500 mL reagent water, add 65 g sodium hydroxide and 6.0 g tetrasodium ethylenediamine tetra-acetic acid. Dissolve and dilute to volume. Store in plastic at room temperature.

6.9 **Preparation of Standards**

6.9.1 **Stock 1000 mg P/L Calibration Standard:** Dry a small amount of potassium dihydrogen phosphate (KH_2PO_4) at 105°C to constant weight. In a 1-L volumetric flask, dissolve 4.394 g of dried reagent in about 500 mL of reagent water. Add 1.0 mL of H_2SO_4 (conc.) and dilute to the mark. Store at 4°C.

6.9.2. **Working Calibration Standards:** Prepare standards over the range of analysis. For the working range of 0 - 10 mg P/L, the following standards may be used:

mL of 6.9.1 Stock diluted to 1 liter	Concentration mg P/L
0.00	0.00
0.50	0.50
1.00	1.00
2.50	2.50
5.00	5.00
7.50	7.50
10.0	10.0

6.9.3 **Stock 100 mg P/L Calibration Standard:** Dry a small amount of Adenosine-5-Monophosphoric Acid Disodium salt, ($C_{10}H_{12}N_5O_7PNa_2 \bullet 2H_2O$, F.W.-427.236 g/mole, Fluka) at 105°C to constant weight. In a 1-L volumetric flask, dissolve 1.3793 g of the dried reagent in about 500 mL of water. Add 1.0 mL of $H_2SO_{4(conc.)}$ and dilute to the mark. Store at 4°C. The spike is prepared by adding 1.00 mL of this stock to 32 mL of sample.

6.9.4 **Working Control Standards:** The following concentrations are typical:

QC Type	mL Control Stock Standard 6.9.3 diluted to 1 liter	Concentration mg P/L
Low Check Standard (CL)	20.0	2.00
High Check Standard (CH)	80.0	8.00

7.0 PROCEDURE

7.1 Soak 50-g capacity porcelain drying dishes, mortars, pestles, digestion tubes, Teflon-lined caps and utensils in 10% sulfuric acid for one hour. Rinse three times in reagent water and dry at 105°C. Take the porcelain drying dishes from the oven and place them in a desiccator to cool to room temperature.

NOTE: *Do not use commercial detergents on dishes, glassware, and utensils prior to the acid soak, as commercial detergents may contain contaminating phosphates.*

7.2 Take the mass in grams of an empty porcelain drying dish. Record this and the dish identification on the Sample Preparation Log Sheet in the column labeled "Dish Weight."

7.3 Fill each porcelain drying dish to capacity. Record the mass of the filled porcelain drying dishes on the Sample Preparation Log Sheet in the column labeled "Weight of Dish and Sample Before Drying." Place samples in the oven at 105-110°C overnight or for no less than eight hours. Then place the samples in the desiccator until cooled to room temperature. Weigh and record the dried sample weight on the Sample Preparation Log Sheet in the column labeled "Weight of Dish + Dry sample."

7.4 Place one sample in the oven again at 105-110°C for an hour. Remove the sample from the oven and place in a desiccator until cooled to room temperature. Weigh and record on the Sample Preparation Log Sheet in the column labeled "Weight of Dish and Sample After Drying an Additional Hour."

NOTE: *In the event that the mass difference between the overnight drying weight and the drying weight plus one hour is greater than 0.5000 grams, repeat step 7.4.*

7.5 Take the whole dried mass from the porcelain drying dish and place it in a grinding mortar. Use the pestle to homogenize and reduce the dried sample into a very fine powder.

7.6 From the dried powdered sample, take a 0.100 - 0.150 g aliquot. On the Sample Preparation Log Sheet, record the true mass of this aliquot in the column labeled "Weight of Dry Sample in Digestion Tube." Transfer the sample aliquots to the digestion tube. Securely cover each tube with a pre-cut 2-inch by 2-inch square of aluminum foil, shiny side up. Place tubes in an all metal tube rack. Place the rack of foil covered tubes inside the pre-heated ash furnace at 425°C. Ash the samples for no less than eight hours. Slowly allow the oven to cool to 75°C before removing the rack of ashed samples. Allow the rack of tubes to cool in a hood to room temperature.

7.7 Add 32.0 mL of DI water and 8 mL of digestion solution **6.3** to the each tube. Cap the tubes to a finger tightness. Place in a metal tube rack.

7.8 To the cleansed dried tubes, add 32.0 mL of working calibration standards, control standards and blanks. Add 8 mL of digestion solution **6.3** to the each tube. Cap the tubes to a finger tightness. Place in a metal tube rack.

7.9 Autoclave the prepared rack at 121°C for 45 minutes. Allow samples to cool to room temperature before analysis.

7.10 Follow the Lachat Procedural SOP for analysis.

8.0 CALCULATIONS

8.1 Results are yielded directly in mg P/L.

8.2 Calculation of Total Phosphorous in Sediment.

$$TP = \frac{C_P \times 0.032}{m}$$

where:

TP = Total phosphorous in sediment (mg P/g of dry sample)
C_P = Concentration of phosphorous from instrument (mg P/L)
m = Weight of dry sediment sample (g)

9.0 QUALITY CONTROL

9.1 The minimum acceptable correlation coefficient (r) is 0.995.

9.2 The following items are required with the minimum frequency indicated.

QC Sample Type		Frequency	Acceptance Criteria
External	Field Duplicate (FD1)	Approximately 10% of samples collected in duplicate	*
Internal	High Check Standard (CH)	At the beginning & end of each batch or 1 per 20 samples, whichever is more frequent	8.00 ± 0.80 mg/L
	Low Check Standard (CL)	At the beginning & end of each batch or 1 per 20 samples, whichever is more frequent	2.00 ± 0.20 mg/L
	Reagent Blank (RB)	At the beginning & end of each batch or 1 per 20 samples, whichever is more frequent	0.00 ± 0.10 mg/L
	Lab Duplicate (LD1)	1 per 20 samples	RPD < 20%
	Round Robin Standard Lab Sediment	Determined by the lab	*
	Matrix Spike (MS)	During each batch or 1 per 40 samples, whichever is more frequent	100% ± 20%

* Acceptance criteria for these QC samples is based on laboratory performance

10.0 WASTE DISPOSAL AND SAFETY PRECAUTIONS

10.1 Because the waste generated from this analytical procedure is very caustic, always wear polypropyl gloves, safety glasses.

10.2 All wastes from this analysis must be poured into the yellow acid waste stream.

10.3 Excess NaOH-EDTA solution must be poured into the blue basic waste stream.

***NOTE**: Do not pour any wastes from this procedure down the laboratory drains. The acid content will erode the plumbing.*

11.0 PREVENTIVE MAINTENANCE

Prescribed maintenance is detailed in the Lachat Procedural SOP.

12.0 TROUBLESHOOTING

12.1 Persistent baseline drift can be eliminated by cleaning the instrument manifold tubing with sodium hydroxide - EDTA rinse not longer than 5 minutes. If the baseline continues to drift after cleaning, replace all the instrument manifold tubes.

12.2 Negative peaks are the result of matrix differences between the samples and the carrier solution or reagent contamination. If reagent contamination is suspected, freshly prepare all reagents and add correct amounts of digestion solution to the standards and samples. Redigest any suspect standards or samples.

12.3 Tiny bubbles tend to develop in the heated loops of the instrument and become trapped in the flow cell, giving erroneous sample measurements or showing a very noisy baseline. Be sure that all reagents pumped through the manifold tubing have been thoroughly degassed with helium. Prior to analysis, thoroughly rinse the manifold tubing with degassed reagent water before pumping the reagents to establish a baseline. Be sure that all tubing connections are secure without restricting fluid flow.

13.0 REFERENCES

13.1 Lachat Instruments, Method Number 10-115-01-1-E, Total Phosphorous in Persulfate Digests. Revision Date May 1992.

13.2 Lachat QuikChem AE Operating Manual.

SAMPLE PREPARATION LOG SHEET

Sample and Dish ID	Dish Weight (g)	Weight of dish and sample before drying (g)	Weight of dish and sample after drying (g)	Weight of dish and sample after drying an additional hour (g)	Weight of dry sample in digestion tube (g)